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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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ABSTRACT

Since the July 1, 1995, QPR which reported implantation of passive iridium and silicon microprobes into the cat cortex, a total of 62 passive iridium microelectrodes were implanted into cat cortex for periods of 2 and 24 hours, and 1 month. Implantation parameters included manual and stereotaxic (slow vs. rapid) approaches, pressure foot (with or without localized cooling of the cortex) and pentobarbital anesthesia with or without hypocapnia. In addition, microelectrode tip configurations consisted of beveled tips and 1, 3 and 6 μm diameter rounded tips. The results showed no advantage of any tip configuration in obviating hemorrhage and, in fact, 34 of the 35 short duration implants (2 and 24 hours) sustained hemorrhages. In a significant number of implants, the smaller diameter tips produced some of the largest hemorrhages. Cavitations and vascular changes were never found. One month after implantation, gliosis and connective tissue sheaths were invariably present. Vascular hypertrophy and hyperplasia were commonly found and several implant sites contained wide areas of scarring, suggesting that these were the sites of earlier hemorrhages. Cavitations were present at only one implant site one month after electrode implantation.

INTRODUCTION

Ideally, intracortical microelectrodes should easily penetrate the pia and the underlying parenchyma in as atraumatic manner as possible. During insertion, they should inflict minimum damage to the parenchymal microcirculation by excessive stretching or shearing forces. This report describes a continuation of our QPR #2, in which we evaluate the extent of trauma exerted by single microelectrodes having one of 4 tip configurations. The microelectrodes were inserted either manually with a forceps or at various speeds using stereotaxic apparatus. We also evaluated the effects of hypothermia, hypoxia and different anesthetics for the induction of cortical vasoconstriction directed at decreasing the cross-sectional area of vasculature encountered by the microelectrodes. The incidence and extent of microhemorrhages, connective tissue scarring, etc., around electrode tracks were assessed in both acute (2-24 hr.) and chronic (1 month) animals.

MATERIALS AND METHODS

Fifteen young adult cats of either sex were used in this series and a total of 62 electrodes were implanted (Tables 1 and 2). These electrodes consisted of pure iridium shafts having either facets or tapering to a final diameter of 1, 3 or 6 μm. The parameters used in the study included: manual (hand-held tweezers) or stereotaxic (slow vs. rapid) insertion with or without a pressure foot (in an attempt to obviate brain movement during implantation). In some instances, near frozen saline was added to the cavity of the pressure foot with the intent of constricting cortical blood vessels.

Preanesthesia was effected with Ketamine/Acepromazine atropine followed by induction of anesthesia with Pentothal and maintained with Halothane, oxygen and nitrous oxide. In a few cases, Pentobarbital was used to induce cerebral vasoconstriction. For the same reason, hypocapnia was induced in 3 cats by increasing the respiration to about 50 to 60/min. for 11 to 40 minutes.

All iridium microelectrodes were 1.5 mm long, 50 μm in diameter and coated with 2 layers of Epoxylite. The tops of the microelectrodes were embedded in an epoxy matrix which was 2.5 mm in diameter and 0.5 mm thick. The four types of tip configuration included a faceted tip and rounded tips with diameters of 1, 3 and 6 μm.

The skull was exposed in the parietotemporal area and craniectomies were carried out bilaterally to expose the gyrus suprasylvius. A "U"-shaped dural flap was fashioned over the gyrus suprasylvius and the individual electrodes implanted on the crown of the gyrus. The two electrodes on each hemisphere were implanted in an anterior-posterior pattern, about 3 to 4 mm apart (Fig. 1). The dura was reapproximated with continuous sutures and the area closed in layers. In the last 2 animals (131-132) the micro-electrodes were meticulously aligned with the shaft of the vacuum holder in 2 orthogonal planes separated by a 90° using a filar ocular inserted into the surgical microscope. Such alignment assures against slashing movement of the electrode during insertion

TABLE 1

IMPLANTS: IC-118 - 132

2 HR.	**IC #**	**# OF IMPLANTS**
Manual, Pentobarbital	127	2
Manual, Pentobarbital, Hypocapnia	127	3
24 HR.		
Manual, Pentobarbital, Hypocapnia	128	4
	129	4
Manual, Pressure Foot With Cooling	125	4
	126	4
Stereotaxic, Slow Pressure Foot Room Temp.	123	4
Stereotaxic, Rapid, No Pressure Foot	118	4
	119	4
Stereotaxic, Rapid, Pressure Foot with Cooling	124	2
1 MO.		
Manual	131	2
Stereotaxic, Slow	132	4
Stereotaxic, Rapid	120	3 (1 lost)
	121	4
	122	4
	131	2
	132	4
Stereotaxic, Rapid, Hypocapnia	130	4

TABLE 2A

IC #	TIP CON-FIG.	METHOD OF INSERTION	MISC.	IMPLANT DURATION (HRS)	LOCA-TION	SURGERY (OBSERVATIONS)	AUTOPSY (OBSERVATIONS)	HISTOLOGY		
								MISC.	NEURONS	HEMORR.
127	1 µm	M	Pento.	2	Rt. Post.	N	Hemorrhage at entry site	Neutro. Edema	N	+++
127	3 µm	M	Pento.	2	Rt. Ant.	N	N	Neutro.	+ Shrunken Hyperchr.	+++
127	6 µm	M	Pento. Hypocap.	2	L.. Ant.	N	Hemorrhage at entry site Hemorrhage (?) on shaft	Edema	+ Shrunken Hyperchr.	+++
127	Faceted	M	Pento. Hypocap.	2	L. Post.	N	N	Track Obscured	Shrunken Hyperchr.	+
128	1 µm	M	Pento. Hypocap.	24	Rt. Ant.	Electrodes moved during dural closure	Hemorrhage at edge of matrix	Neutro.	N	+++
128	3 µm	M	Pento. Hypocap.	24	Rt. Post.	Electrodes moved during dural closure	Hemorrhage around entry site	Neutro.	N	+++
128	6 µm	M	Pento. Hypocap.	24	L. Ant.	Electrodes moved during dural closure	Hemorrhage around entry site	Neutro. Obscured track	Obliterated	+++
128	Faceted	M	Pento. Hypocap.	24	L. Post.	Electrodes moved during dural closure	Hemorrhage around entry site	Neutro.	N	+++
129	1 µm	M	Pento. Hypocap.	24	Rt. Ant.	N	Hemorrhage around entry site	Neutro.	N	+++
129	3 µm	M	Pento. Hypocap.	24	Rt. Post.	N	N	Neutro.	N	+++
129	6 µm	M	Pento. Hypocap.	24	L. Ant.	N	Hemorrhage around entry site	Neutro.	N	+++
129	Faceted	M	Pento. Hypocap.	24	L. Post.	N	N	———	N	+++

Cav = Cavitation
Hyperchrom. = Hyperchromic
Hypocap. = Hypocapnia
Hyperplas. - Hyperplasia
Hypertroph. - Hypertrophy
M = Manual Insertion
Neutro. = Neutrophils

PF = Pressure Foot, no cooling
PFC = Pressure Foot, with cooling
Pento. = Pentobarbital
SS = Stereotaxic, slow
SR = Stereotaxic, rapid

\+ = Slight
++ = Moderate
+++ = Marked

TABLE 2B

IC #	TIP CON-FIG.	LOCA-TION	METHOD OF INSERTION	IMPLANT DURATION (HRS)	SURGERY (OBSERVATIONS)	AUTOPSY (OBSERVATIONS)	HISTOLOGY		
							MISC.	NEURONS	HEMORR.
125	1 µm	Rt. Post.	M; PF	24	Hemorr. at point of insertion	Hem around entry site. Subdural hemorr around and under matrix. Hem (?) on shaft	———	+ Flat & Hyperchr.	++
126	1 µm	Rt. Post.	M; PF	24	N	Small Subdural hemorr. medial to electrode and around electrode entry site	———	+++ Flat	+++
125	3 µm	Rt. Ant.	M; PF	24	N	Small hemorrhage around entry site Hemorrhage (?) on shaft	Neutro. at entry site	+ Flat	+++
126	3 µm	Rt. Ant.	M; PF	24	N	Hemorr. around electrode entry site	++ Neutro. around track	++ Flat	++
125	6 µm	L. Post.	M; PF	24	Cortical hemorr. during suturing of dura	Small hemorr. medial to electrode entry site	———	+ Flat	+++
126	6 µm	L. Post.	M; PF	24	Dural hemorr. during suturing	Hemorrhage medial to electrode site	———	+ Flat & Hyperchr.	++
125	Faceted	L. Ant.	M; PF	24	Cortical hemorr. during suturing of dura	Hemorr. (?) on shaft. Sm. hemorr. around entry site. Sm. hemorr. lat. to electrode site		++ Flat	+++
126	Faceted	L. Ant.	M; PF	24	Dural hemorr. during suturing	Hemorr. at entry site Hemorr. (?) on shaft	Neutro. at entry	+ Flat	++
123	1 µm	Rt. Post.	S; PF	24	Brain excursion not obviated by pressure foot	Hemorr. around and beneath matrix Hemorr. (?) on shaft	———	+++ Flat	+++
123	3 µm	Rt. Ant.	S; PF	24	Non-release of electrode holder. Brain excursion not obviated by pressure foot.	Hemorr. around matrix Hemorr. (?) on shaft	Neutro.	++ Flat	+++
123	6 µm	L. Post.	S; PF	24	Non-release of electrode holder. Brain excursion not obviated by pressure foot.	Cortical hemorr. post. to electrode	Neutro.	+++ Flat	+++
123	Faceted	L. Ant.	S; PF	24	Non-release of electrode holder. Brain excursion not obviated by pressure foot.	N	+ Neutro.	++ Flat	+++

TABLE 2B (CONT.)

IC #	TIP CON-FIG.	LOCA-TION	METHOD OF INSERTION	IMPLANT DURATION (HRS)	SURGERY (OBSERVATIONS)	AUTOPSY (OBSERVATIONS)	HISTOLOGY		
							MISC.	NEURONS	HEMORR.
118	1 µm	Rt. Ant.	SR	24	N	N	Neutro. in Hemorr.	++ Flat	++
118	1 µm	Rt. Post.	SR	24	N	Hemorr. around entry site	Neutro. in Hemorr.	N	+++
118	3 µm	L. Ant.	SR	24	N	N	Neutro. in track	+ Flat & Hyperchr.	+++
118	3 µm	L. Post.	SR	24	N	Hemorr. around entry site	Neutro. in track	+ Flat	+
124	3 µm	L. Ant.	SR; PFC	24	Stereotaxic insertion incomplete. Completed manually	Hemorr. around entry site	Edema	+++ Shrunken & Hyperchr.	0
119	6 µm	Rt. Ant.	SR	24	N	N	Lost	Lost	Lost
119	6 µm	Rt. Post.	SR	24	N	N	Neutro.	N	+++
124	6 µm	L. Post.	SR; PFC	24	N	Hemorr. around entry site	Edema	+++ Shrunken & Hyperchr.	+++
119	Faceted	L. Ant.	SR	24	Pial Nick	Hemorr. around entry site	Edema Neutro.	N	+++
119	Faceted	L. Post.	SR	24	Pial Nick	Hemorr. under matrix	———	+ Flat	+++

CODE:

M = Manual Insertion
SR = Rapid Stereotaxic Insertion (piston & sleeve)
Hemorr. = Hemorrhage
CAV. = Cavitation
+ = Slight ++ = Moderate +++ = Marked
Hypocap. = Hypocapnia
Hyperchr. = Hyperchromic
Hyperplas. = Hyperplasia
Hypertro. = Hypertrophy
Neutro. = Neutrophils
PF = Pressure Foot, No Cooling
PFC = Pressure Foot, Cooked
Pento. = Pentobarbital

TABLE 2C

IC #	TIP CON-FIG.	LOCA-TION	METHOD OF INSERTION	MISC.	IMPLANT DURATION (1 MONTH)	SURGERY (OBSERV.)	AUTOPSY (OBSERV.)	HISTOLOGY					
								MISC.	NEU-RONS	HEMORR.	GLIO-SIS	VASC. PROLIF.	SHEATH THICKNESS (µm)
131	6 µm	L. Post.	M	—	1 month	Considerable bleeding	N	—	+ Flat	0	+	++Hyperplas.	25-70
131	Faceted	L. Ant.	M	—	1 month	N	N	—	+ Flat	0	++	++Hyperplas.	8-20
120	1 µm	Rt. Ant.	SR	—	1 month	Dura torn	N	—	N	0	++	0	150
120	1 µm	Rt. Post.	SR	—	1 month	Dura torn	Tissue adherent to distal end of shaft	—	+ Flat	0	+++	++ Hypertro.	175
122	1 µm	Rt. Ant.	SR	—	1 month	Not fully inserted; completed manually	N	—	+ Flat	0	++	++ Hypertro.	50-150
122	1 µm	Rt. Post.	SR	—	1 month	Not fully inserted; completed manually	N	—	+ Flat	0	++	++ Hypertro.	50-150
131	3 µm	Rt. Ant.	SR	—	1 month	N	N	—	+ Flat	0	++	++ Hypertro.	15
131	1 µm	Rt. Post.	SR	—	1 month	N		Perivasc. Cuff (Lymph.)	+++ Flat	0	+	+ Hypertro. ++Hyperplas.	10-15
120	3 µm	L. Post.	SR	—	1 month	N	N	—	+ Flat	+ near track (? movemnt.)	++	+ Hypertro.	30-40
120	3 µm	L. Ant.	Lost	Lost	Lost	Lost	Lost	Lost	Lost	Lost	Lost	Lost	Lost
122	3 µm	L. Ant.	SR	—	1 month		N	—	N	0	++	0	100-250
122	3 µm	L. Post.	SR	—	1 month	Not fully inserted; completed manually	N	Siderophages	+ Flat	0	++	0	40

TABLE 2C (CONT.)

IC #	TIP CON-FIG.	LOCA-TION	METHOD OF INSERTION	MISC.	IMPLANT DURATION (1 MONTH)	SURGERY (OBSERVATIONS)	AUTOPSY (OBSERVATIONS)	HISTOLOGY					
								MISC.	NEU-RONS	HEMORR.	GLIOSIS	VASC. PROLIF.	SHEATH THICK. (µm)
121	6 µm	L. Ant.	SR	—	1 month	2 penetrations	Small dural hemorrhage	—	+ Flat	0	+	0	100
121	6 µm	L. Post.	SR	—	1 month	N	? Old hem. on electrode shaft	—	N	0	+++	++ Hyperplas. ++ Hypertrop.	10-35
121	Facet	Rt. Ant.	SR	—	1 month	Rt. Hemis. Depressed	N	—	N	0	++	0	70
121	Facet	Rt. Post.	SR	—	1 month	Rt. Hemis. Depressed Penetration at angle	N	—	N	0	++	+++ Hypertro.	90
130	6 µm	L. Post	SR	—	1 month	N	Electrode inadv. pulled out	—	+ Flat	0	+++	0	10-15
130	Facet	L. Ant.	SR	—	1 month	N	Electrode inadv. pulled out. Brownish film on shaft	—	N	0	++	0	25-30
130	1 µm	Rt. Ant.	SR	Hypo-cap.	1 month	3 penetrations. Con-siderable bleeding during suturing	Electrode inadvertently pulled out. Brown film on shaft	—	+ Flat (1 side)	0	++	0	15-125
130	3 µm	Rt. Post.	SR	—	1 month	Considerable bleeding during suturing	Electrode advertently pulled out. Colorless tissue over tip	—	+ Flat	0	+	0	5-15
132	1 µm	Rt. Post.	SR	—	1 month	2 penetrations (non-release of vacuum holder)	N	Scar near tip	+++ Flat	0	++	++ Hypertro.	10
132	3 µm	Rt. Ant.	SR	—	1 month	N	N	Perivasc. Cuffing (Lympho.)	+ Flat	0	++	0	8-10
132	6 µm	L. Lat.	SR	—	1 month	Moved during suturing of dura	Electrode inadvertently pulled out	—	++ Flat	0	++	+ Hypertro.	To 125
132	Facet	L. Med.	SR	—	1 month	N	Electrode inadvertently pulled out	Scar near tip	+ Flat	0	++	0	15

RESULTS

Because of the large variety of parameters used, it was considered easier to correlate the surgical, autopsy and histologic findings by combining them for each category of parameters.

I. **Two-hour implant duration**. Stereotaxic frame-mounted axial introductor (SMAI). Slow insertion. Pentobarbital anesthesia with normocapnia or hypocapnia was used. A single animal (IC-127) in this category received implants of 4 different types. The 1 and 3 μm electrodes were inserted during pentobarbital anesthesia and normocapnia while the 6 μm and faceted electrodes were inserted using the same anesthesia but during hypocapnia. At autopsy, a small hemorrhage was present at 2 electrode entry sites (1 and 6 μm diameter tips). A brownish substance (presumably blood) was present on the shaft of the 6 μm electrode. Histological examination revealed hemorrhages up to 960 μm in greater dimension adjacent to all tracks. Shrunken, hyperchromic neurons near the track were present near all but the 1 μm electrode track. Due to the short duration of the implantation, vascular changes (hyperplasia or hypertrophy), cavitation or gliosis were not present.

II. **Twenty-four hour implant duration.** A total of 30 electrodes were implanted in 8 cats. The variables employed included the use of a pressure foot with manual or SMAI (slow or rapid) penetration, manual insertion with a combination of pentobarbital anaesthesia and induced hypocapnia, and finally, rapid stereotaxic insertion without a pressure foot. The short duration of the implants precluded the development of vascular changes (hyperplasia or hypertrophy), cavitation or gliosis. For technical reasons, the track of 1 of the 27 implant sites (IC-118, 6 μm tip)was not recovered.

A. Manual insertion. No pressure foot (2 cats, 4 implants each, IC-128,129). Pentobarbital anesthesia and hypocapnia were used. One cat (IC-128) showed visible movement of the implanted electrodes during reapproximation and suturing of the dural flap over the operative site. At autopsy, 2 electrode sites in each animal showed a small hemorrhage encircling the electrode entry site. Histological examination showed that all electrode tracks were associated with hemorrhages. The sizes of the hemorrhages were not correlated with the size or shape of the electrode tip. The largest hemorrhages were 1 mm or larger in greater dimension and present at various

depths along the track (Fig. 2). Neutrophils were commonly present in the leptomeninges near the track and within the hemorrhagic areas. Neurons near every track appeared normal.

B. Manual insertion. Pressure foot with cooling (2 cats, 4 implants each, IC-125,126). At surgery, the pressure foot failed to obviate cortical movement at the implant sites. Dural hemorrhage occurred at two operative sites on each animal during reapproximation and suturing. One additional site showed a hemorrhage at the point of electrode insertion (IC-125, 1 μm tip). At autopsy, 5 of the 8 electrode implant areas showed a hemorrhage around the entry site. These showed no obvious pattern relative to the type of electrode tip. Microscopic examination showed that neurons near all tracks were mechanically flattened (Fig. 3) but appeared hyperchromic at only 2 sites. Paradoxically, the latter were present along one larger (6 μm tip) and the smallest (1 μm) electrode sites. Hemorrhage was present at all sites, and some of the smaller hemorrhages (200 to 300 μm) were present at track with the larger tips (6 μm and faceted) while a 3 μm tipped electrode caused a 1,500 μm long (IC-125, 3 μm tip) hemorrhage. Thus, no consistent pattern of hemorrhages was associated with a particular tip configuration.

C. SMAI, slow penetration. Uncooled pressure foot (1 cat, 4 implants, IC-123). As mentioned above, the pressor foot did not prevent movement of the brain relative to the electrodes. The brain movement was due to the cardiac pulsations. At autopsy, 3 of the 4 sites showed cortical hemorrhage around or beneath the matrix, and the 1 and 3 μm electrodes showed a thin, brownish film adherent to the electrode shaft. Probably, this was a layer of blood from a ruptured blood vessel during implantation. The histological examination showed numerous neutrophils infiltrating the leptomeninges near the track and dispersed within hemorrhagic areas. Hemorrhages were prominent along all 4 tracks and reached as much as 550 μm in length. Again, there was no pattern of hemorrhage (relative to electrode tip configuration) observed at

surgery, autopsy or in the histology sections. Neurons near all 4 tracks were mechanically flattened but were not hyperchromic, and appeared otherwise normal.

D. SMAI, rapid penetration. No pressure foot. (2 cats, 4 implants each, IC-118,119). For technical reasons, one (3 μm tip) electrode track was lost to the study. Aside from a pial nick at 2 of 8 sites, surgery was unremarkable. At autopsy, hemorrhage was present around 3 electrode entry sites (faceted tip and 1 μm and 3 μm conical tips). Another faceted electrode sites showed a cortical surface hemorrhage encircling the electrode matrix. Histologically, neurons near the tracks appeared normal near 3 tracks while at the other sites, they were mechanically flattened with shrunken, hyperchromic profiles at two of these sites. Hemorrhage was present at all tracks and ranged in size as follows: 1 μm tips: 270-650μm; 3 μm tips: 150 to 1,000 μm; 6 μm tips: 900 μm; faceted tips: 500-550 μm. It was obvious that the smaller diameter tips did not confer immunity from hemorrhages and, in fact, produced some of the largest hemorrhages (Fig. 4).

E. SMAI, rapid insertion. Pressure foot and cooling (One cat, two implants, IC-124). At surgery, one 6 μm electrode and another with a 3 μm diameter tip were used. The latter was not completely inserted with the stereotaxic device and consequently was pushed in manually. At autopsy, a small hemorrhage was present around both electrode entry sites. The histologic examination showed an edematous zone around both tracks and numerous neurons near the track were shrunken and hyperchromic. Only one of the tracks (6 μm electrode) was associated with a hemorrhage and this was 450 μm in size.

F. Morphometry. In one animal, IC 112, we used our global lab image analysis technique (QPR3) to quantitate the vascular density of the cortex (McCreery et.al, 1996).

III. **One month implant duration.**

A. Manual insertion (1 cat, 2 implants, IC-131). At surgery, considerable bleeding occurred at the posterior electrode, probably due to improper electrode alignment. The autopsy findings were unremarkable. The histological studies showed a mild to moderate gliosis around the track and a few nearby neurons were mechanically flattened. Moderate hyperplasia was present near both tracks. The sheath surrounding the tracks varied from 20 to 70 μm in thickness.

B. SMAI, slow penetration (IC-132, 4 sites). At surgery, the failure of the vacuum holder to release the electrode necessitated 2 penetrations at 1 site. During suturing of the dura, 1 electrode was contacted but not dislodged. At autopsy, 2 electrodes were pulled out prematurely due to adherence of the dura to the electrode matrices. Histological examination revealed perivascular cuffing around a vessel near the 3 μm electrode site and a rounded scar near the tips of the 1 μm and faceted electrodes. Neurons near all tracks were mechanically flattened. Moderate gliosis accompanied all tracks and 2 tracks were accompanied by moderate vascular hypertrophy. With one exception, sheath thickness was about 10 to 15 μm. Hemorrhage and cavitations were not present.

C. SMAI, rapid penetration (4 cats, 17 implants. IC-120,121,122,131,132. One track lost to the study.). At surgery, two electrodes were not fully inserted by the stereotaxic device and necessitated manual assistance. In one instance, it was necessary to make two penetrations to effect complete implantation. One hemisphere was found depressed and an electrode was inserted into this hemisphere at an angle (IC-121).

At autopsy, one electrode (IC-120, 1 μm tip) showed tissue adherent to the tip. Two shafts (IC-121, 6 μm tip and IC-130, faceted tip) were covered with a film of unidentified brownish tissue, possibly blood.

Histological examination revealed neuronal flattening near 7 of the 9 tracks having tips of 1 and 3 μm diameter (Fig. 5). Conversely, only 2 of 6 tracks with 6 μm or faceted tips showed mechanical distortion of neurons. Despite the distortion, the neurons were not hyperchromic or shrunken. Moderate numbers of glial cells were present around most tracks. In a few other instances, the gliosis was either slight or marked. There was no clear cut pattern of glial cell proliferation relative to tip configuration. The same was true for vascular hyperplasia and hypertrophy. About 50% of the tracks of electrodes with 1 and 3 μm tips showed the vascular phenomenon. Of the 6 μm and faceted tip implants, one of three sites in each category showed the change.

The sheath thickness around the tracks in this group ranged from 15 to 250 μm and averaged 90 μm. The only hemorrhage in this entire series was present along the track in IC-120 (3 μm tip) and appeared to be a fresh hemorrhage extending from a depth of 750 to 1,500 μm. In a few instances, connective tissue scars near the tip (Figs. 5 and 6) or higher up along the track undoubtedly represent sites of earlier hemorrhage and subsequent resolution as a scar, devoid of neurons or other neural elements.

D. SMAI, rapid injection, hypocapnia (1 cat, 2 implants, IC-130). Due to malfunction of the stereotaxic device the 1 μm tipped electrode required 3 penetrations. At autopsy, this same electrode was covered by a thin, brownish film of tissue, probably blood. The 3 μm tipped electrode retained a fragment of colorless tissue on the tip. The histologic examination showed flattening of neurons accompanying the 1 μm electrode but those near the 3 μm electrode track appeared normal. Mild to moderate gliosis accompanied the tracks. The sheath around the tracks varied from 15 to 125 μm in thickness.

E. GFAP staining for astrocytes. In some instances, comparison pairs of micrographs (Figs. 7A&7B and 8A&8B) were taken in which the micrographs

in 7A and 8A are routinely stained sections (H&E or Nissl) and their adjacent sections (B) are stained for GFAP (glial fibrillary acidic protein). Both resting and activated astrocytes were specifically stained. In a few instances,

F. Morphometry. The vascular density of the cerebral cortex of IC-112 was 363/mm². From this and the volume of the microelectrode it was calculated that the number of vessels encountered by the microelectrode inserted to a depth of 1.5mm was 27 (Table 3)

TABLE 3

MORPHOMETRY OF IMPLANT SURROUND

Vascular density	363/mm²
Total space occupied by blood vessels	2%
Range of vessel diameters (Not corrected for shrinkage)	3- 35 mm
Vessels Encountered by a 50 mm diameter electrode inserted to a depth of 1.5 mm	27

SUMMARY

I. **Two- and 24-hour implant durations.** With respect to electrode tip configuration and hemorrhages, none of the 4 tip types obviated rupture of cortical blood vessels. In fact, of the 35 implants, only one, a 3 μm diameter tipped electrode, was successfully implanted without hemorrhage. A number of the smallest tipped electrodes produced some of the largest hemorrhages and vice versa. Cavitations, vascular changes (hyperplasia and hypertrophy) were absent. Gliosis was never present.

With the exception of 1 of 8 sites where massive bleeding obliterated nearby neurons, all of the remaining 7 sites, where the electrodes were implanted manually, showed undistorted, normal-appearing neurons. All other methods of implantation resulted in significant neuronal flattening. As early as 2 hours after implantation, neutrophilic infiltration into the hemorrhagic areas and leptomeninges was a common finding. By 24 hours, the phenomenon was almost universally present

In the 3 instances of edema adjacent to the tracks in the 2- and 24-hour implants, it is significant that 2 of these occurred in the same animal (IC-124) in which both a pressure foot and cooling of the cortex were used. Further, the presence of shrunken neurons near these same implants supports the concept that the marked cooling of the cortex with nearly frozen saline slush was damaging.

II. **One month implant duration.** With a single exception of fresh hemorrhage along the track, hemorrhages were not present. Areas of connective tissue and gliosis adjacent to the tracks undoubtedly represent sites of earlier hemorrhage with subsequent resolution in the form of scarred areas. Twelve of the 19 tracks were

accompanied by mechanically flattened neurons. This was surprising in view of the relatively long implant period during which time the neurons would have been expected to resume their normally rounded shape.

Aside from a single instance of unexplained perivascular cuffing by lymphocytes near the track, there was no evidence of inflammation. Cavitation was not present. Gliosis was universally present along the track and around the tip. Along 10 of the 19 tracks, vascular hyperplasia and/or hypertrophy were prominent, a phenomenon consistently seen in previous series. The sheath lining the track averaged 90 μm in thickness.

The GFAP stain allowed distinct visualization of astrocytes, both resting and activated. As commonly understood and demonstrated here, resting astrocytes and their processes are components richly represented on the underside of the pial membrane. The GFAP stain clearly shows the proportion and distribution of the sheath represented by activated astrocytes as compared to the remaining, connective tissue, component.

DISCUSSION

The present data substantiate and extend our recent observations (QPR 1 and 2) that acute intracortical electrode implantation is accompanied by microhemorrhages at all depths along the track. The evaluation of the effect of anesthetics and agents of vasoconstriction, added in this study, also failed to decrease the incidence of hemorrhages. The use of glial fibrillary acid protein (GFAP) stain has been initiated in this study and will be employed selectively in future studies. This stain is specific for fibrillary proteins in activated astrocytes and enables clear distinction between glial and connective tissue elements around electrode tracks. Gliosis is a clear indicator of the extent of neural damage.

At 24 hours, the hemorrhagic sites appeared as lakes of extravasated RBC's mixed with neutrophils. These sites were frequently seen to have an asymmetric distribution to one

side of the electrode track probably indicative of release of blood under pressure subsequent to the rupture of small arteries. In general, the connective tissue and glial scarring observed at 1 month paralleled the distribution of the hemorrhagic sites seen in the acute (24 hr.) implant sites. Of particular relevance to this study is the fact that large portions of the neuropil (up to 0.2-0.3 mm^2) are obliterated in the acute stage and the void replaced by non-neural tissue after 1 month (connective tissue and glial cells). Of the average of 27 blood vessels encountered by a 1.5 mm microelectrode 50 μm in diameter (Table 3) one or more vessels in virtually every case are either disrupted or penetrated resulting in the histologic findings of this report.

It is encouraging that in the one month implants, numerous viable-appearing neurons were present less than 50μm of the electrode tip. In addition, in previous studies in which similar electrodes were pulsed, activating thresholds as low as 8μA were obtained (Agnew et.al, 1986). An interesting parallel to this report is described by histologic studies of the cerebral cortex of mice following stab wounds with a 26 gauge needle (Wallace and Bisland, 1994). The brains were examined at 1-63 days and stained with NADPH-diaphorase which revealed many microhemorrhages along the needle tracks particularly at the shorter implant periods. The ischemic damage produced by dividing arterioles produced rims of reactive astrocytes containing NADPH-diaphorase that surrounded the lesion within one day of the stab wound.

Conclusions from both this and two previous reports (QPR's 1 and 2) are that the incidence of microhemorrhages is unaffected by tip configuration, speed of insertion or cerebral vasoconstriction during electrode insertion. In addition, implantations in this study were performed by two surgeons. Still to be evaluated are the use of microelectrodes with thinner shafts, (37 rather than 50 μm in diameter), the use of blunt microelectrodes (15μm diameter tips) and study of the role of suturing the dura in the trauma observed (dura will be unsutured in a few acute experiments).

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WORK NEXT QUARTER

This study will be extended to include a series of implants using more blunt 15 μm-tip micro-electrodes. We will also evaluate the role suturing the dura in the production of electrode movement and subsequent scarring around the microelectrodes without suturing the dura in acute experiments.

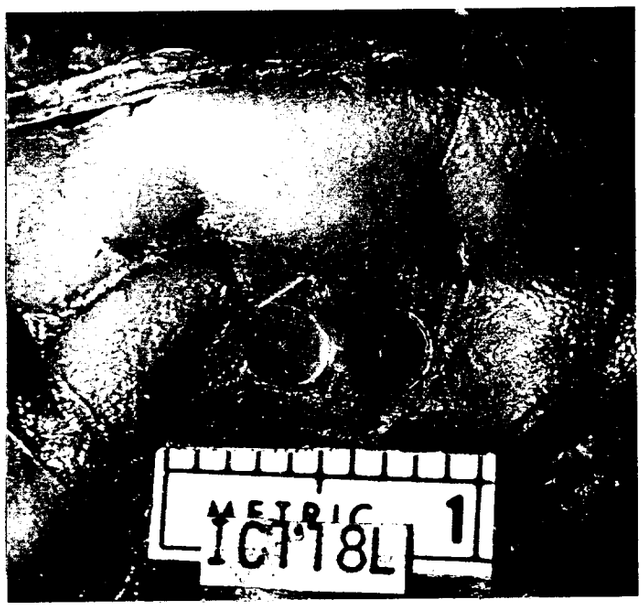

Fig.1 (IC-118). Autopsy view of two electrodes in situ on the crown of the left gyrus suprasylvius. Each electrode has a 3μm diameter tip. The matrix for each electrode is composed of epoxy, 0.5 mm thick. The suture attached to each matrix facilitates handling at surgery.

Fig. 2(IC-128). Coronal section through the track left by a 3μm tipped electrode 24 hours after manual implantation. Pentobarbital anesthesia and hypocapnia were employed to induce vasoconstriction. Despite the small electrode tip and vasoconstriction, bleeding and erosion of the neuropil (arrow heads) along the track (T) were marked. Nissl stain. Bar=500μm. This and all subsequent micrographs were taken from coronal serial section of paraffin-embedded brain cortex.

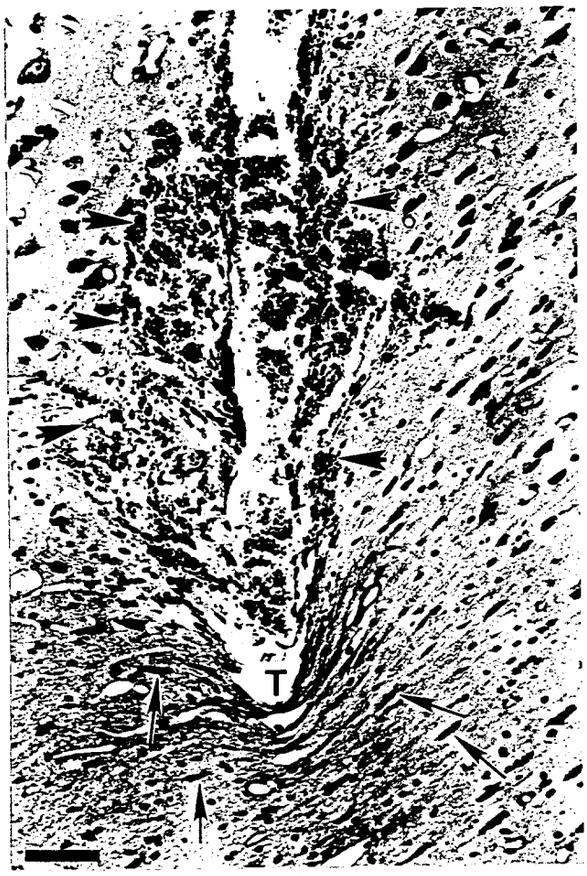

Fig. 3(IC-126). Faceted tip, manual insertion; pressure foot and cortical cooling. Implant duration 24 hours. Nissl-stained section showing the electrode tip site (T) at a depth of 1,300μm. Tissue along each side of the track has been destroyed by hemorrhage (delimited by arrow heads). Note the marked mechanical flattening of several neurons (arrows) below and to the right of the tip area. Bar=150μm.

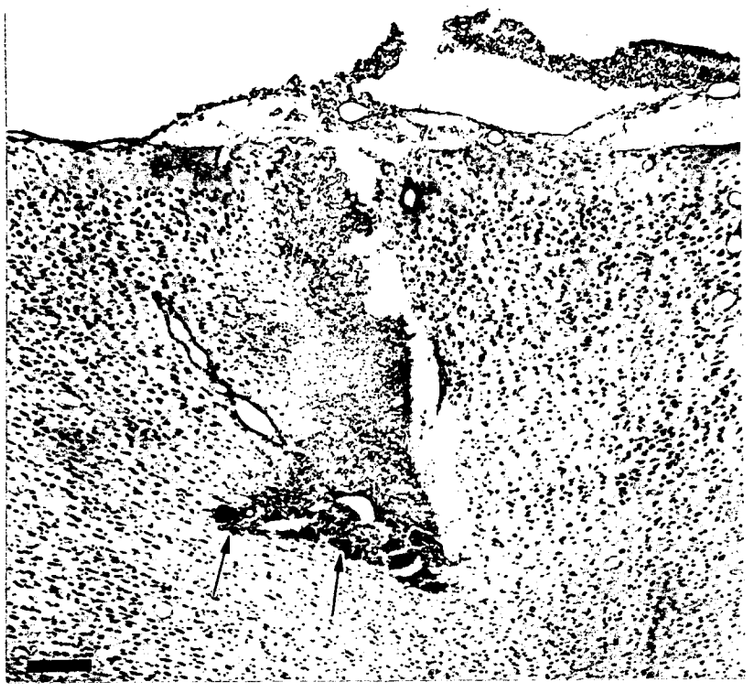

Fig. 4 (IC-118). 1μm tip. Stereotaxic approach, rapid insertion. Implant duration 24 hours. Nissl-stained section through the mid-portion of the obliquely sectioned track. Note the considerable bleeding at both the top and bottom segments of the track. The lower hemorrhage (arrows) extends 1,700μm away from the track and has obliterated all neural elements in the area. The upper hemorrhage has also destroyed much tissue. Numerous neutrophils have invaded the hemorrhage adjacent to the upper part of the track. Bar=500μm

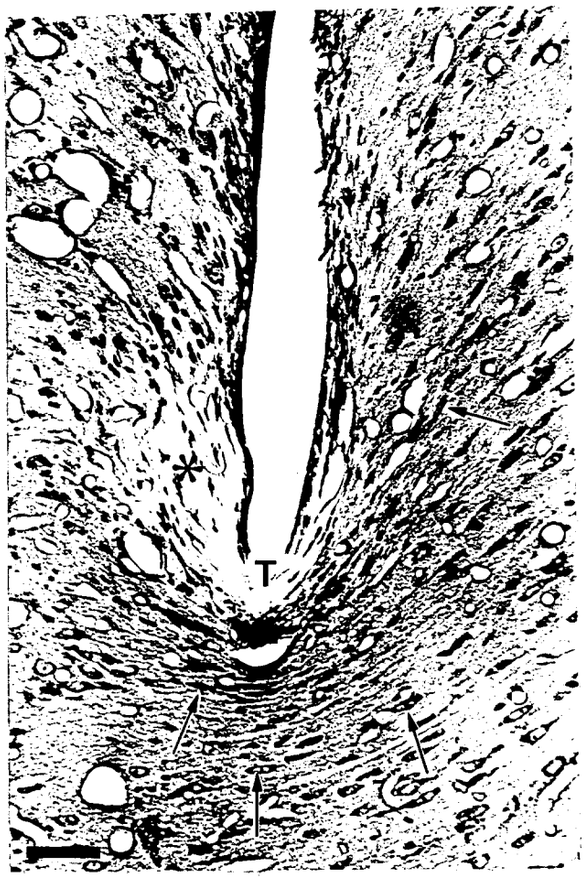

Fig. 5 (IC-120). 1μm tip. Stereotaxic approach, rapid penetration. Implant duration 1 month. Nissl-stained section showing the tip (T) of the track at a depth of 1,500μm. Moderate hypertrophy accompanies the track (left side of micrograph). A scar (*) is present to one side of the tip. Note the numerous mechanically compressed, but apparently viable, neurons (arrows) alongside and below the tip site. Bar=150μm.

Fig. 6 (IC-121). Faceted tip. Stereotaxic approach, rapid injection. Implant duration 1 month. Nissl-stained section showing the tip site (T) at a depth of 1,400μm. Numerous neurons on each side of the tip site and below it are mechanically flattened. Just above the tip site a scar (asterisks) surrounds the track. Several glial cells (dark profiles) closely skirt the edges of the scar. Bar=150μm.

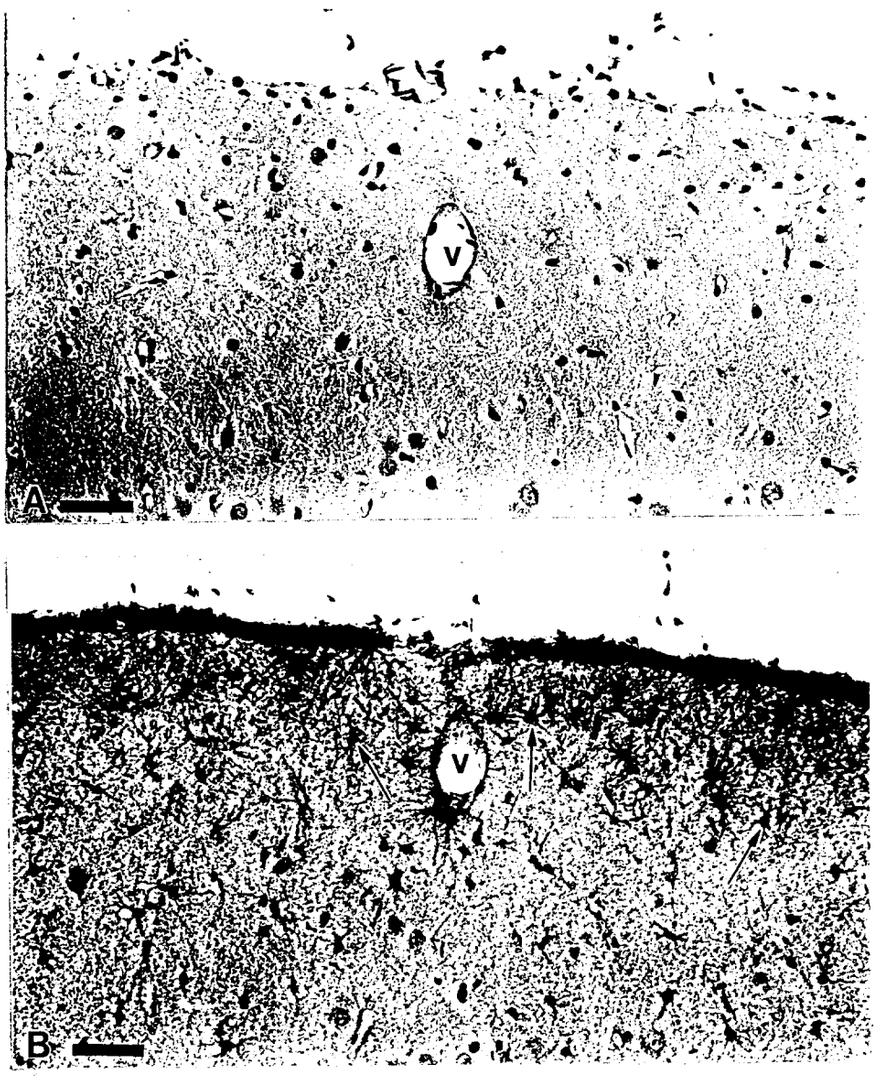

Fig. 7 Control cortex. A. Coronal section stained only with H&E. B. Section immediately adjacent to that shown in A and stained with H&E and GFAP, a filament-specific stain for astrocytes. Note the numerous branched astrocytes (arrows) concentrated below the pia. The underside of the pia (glia limitans) is know to constitute the terminus for many glial fibers and small astrocytes, hence the darkly stained, thickened appearance of the pia here. Astrocytic foot processes are known to abut against blood vessels and the phenomenon is clearly shown here. V=blood vessel. Bar=150μm.

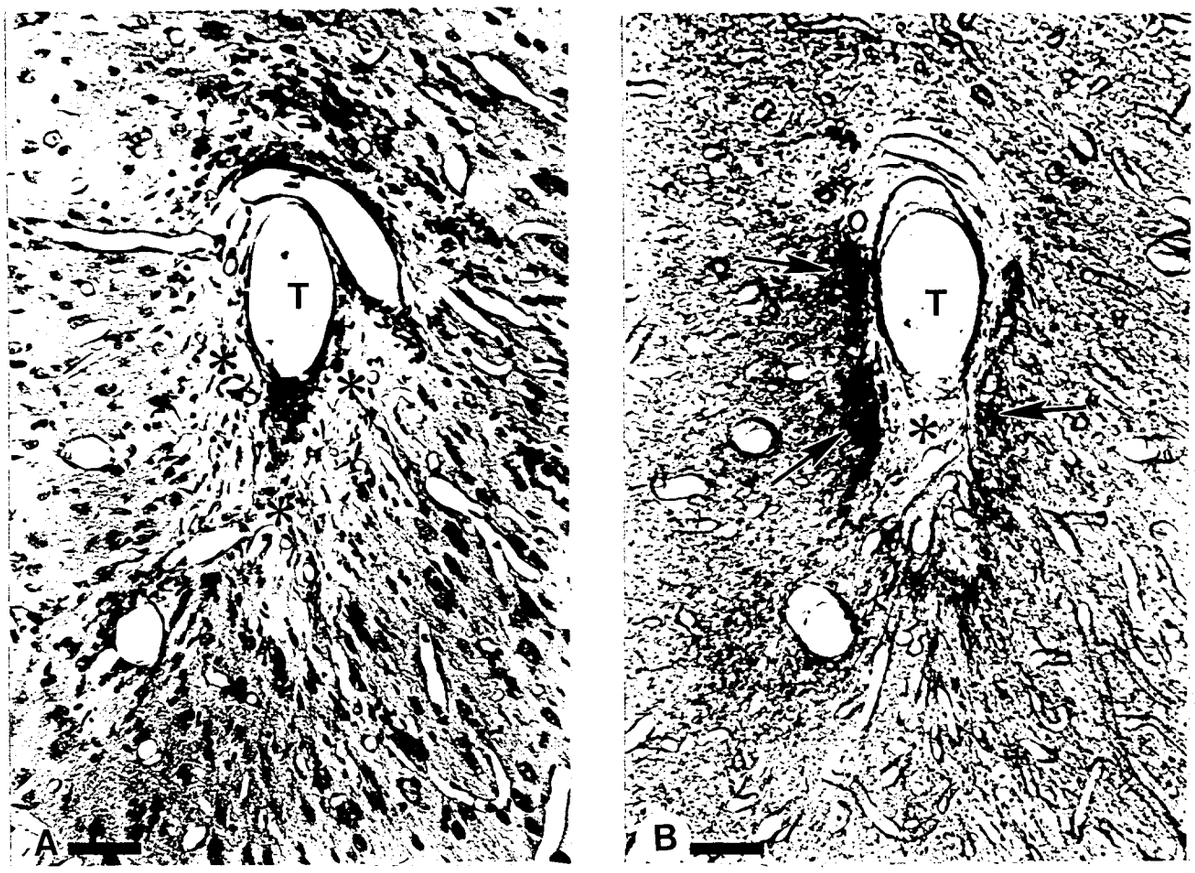

Fig. 8 (IC-121). Faceted tip. Stereotaxic approach, rapid penetration. Implant duration 1 month. Cerebral cortex showing scarring around electrode track (T). A. Nissl-stained section shows scar tissue (*) around and below the track (T). B. Adjacent section stained only for GFAP, a filament specific stain for astrocytes. Note the unstained area (*) below the track is composed of compact connective tissue which, in turn, is skirted by dark staining gliotic-scarring (arrows). Bars=150μm.